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Rongsong Li

Alessandra Adami

Chih-Chiang Chang

Chi-Hong Tseng

Tzung K. Hsiai

See next page for additional authors

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Authors

Rongsong Li, Alessandra Adami, Chih-Chiang Chang, Chi-Hong Tseng, Tzung K. Hsiai, and Harry B. Rossiter

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*Rongsong Li[1], *Alessandra Adami[2,3], Chih-Chiang Chang[4], Chi-Hong Tseng[4], Tzung K. Hsiai[4], Harry B. Rossiter[3,5]

[1]College of Health Science and Environmental Engineering, Shenzhen Technology University, Shenzhen, Guangdong, China; [2]Department of Kinesiology, University of Rhode Island, Kingston, RI; [3]Rehabilitation Clinical Trials Center, Division of Respiratory and Critical Care Physiology and Medicine, The Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, Torrance, CA; [4]Department of Medicine, West Los Angeles VA Healthcare System, University of California, Los Angeles, CA; [5]Faculty of Biological Sciences, University of Leeds, Leeds, United Kingdom

*These authors contributed equally

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*Rongsong Li[1], *Alessandra Adami[2,3], Chih-Chiang Chang[4], Chi-Hong Tseng[4], Tzung K. Hsiai[4], Harry B. Rossiter[3,5]

[1]College of Health Science and Environmental Engineering, Shenzhen Technology University, Shenzhen, Guangdong, China; [2]Department of Kinesiology, University of Rhode Island, Kingston, RI; [3]Rehabilitation Clinical Trials Center, Division of Respiratory and Critical Care Physiology and Medicine, The Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center, Torrance, CA; [4]Department of Medicine, West Los Angeles VA Healthcare System, University of California, Los Angeles, CA; [5]Faculty of Biological Sciences, University of Leeds, Leeds, United Kingdom

*These authors contributed equally

Correspondence

Harry B. Rossiter, PhD

The Lundquist Institute for Biomedical Innovation at Harbor-UCLA Medical Center

1124 W. Carson St., CDCRC Building, Torrance, CA 90254

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Tel: 310 222 8200

Email: hrossiter@ucla.edu

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ABSTRACT

Purpose: Chronic obstructive pulmonary disease (COPD) is associated with altered metabolism and body composition that accompany poor outcomes. We aimed to determine whether metabolic derangements in COPD are associated with skeletal muscle deconditioning and/or physical inactivity, independent of pulmonary obstruction.

Methods: We characterized serum metabolites associated with muscle oxidative capacity or physical activity in 44 COPD patients (FEV_1=61±4%predicted) and 63 current and former smokers with normal spirometry (CON) (FEV_1=93±2%predicted). Medial *gastrocnemius* oxidative capacity was assessed at rest from the recovery rate constant (*k*) of muscle oxygen consumption using near-infrared spectroscopy. Step counts and physical activity (average vector magnitude units (VMU)/min) were measured over 5-7 days using triaxial accelerometry. Untargeted prime and lipid metabolites were measured using liquid chromatography and mass spectrometry.

Results: Muscle *k* (1.12±0.05 vs. 1.68±0.06min^{-1}; *P*<0.0001; d=1.58) and VMU/min (170±26 vs. 450±50 VMU/min; *P*=0.004; d=1.04) were lower in severe COPD (FEV_1<50%predicted, n=14-16) compared with CON (n=56-60). 129 prime metabolites and 470 lipids with known identity were quantified. Using sex as a covariate, lipidomics revealed 24 differentially expressed lipids (19 sphingomeylins) in COPD, consequent to a diminished sex difference of sphingomeylins in COPD (FDR<0.05; n=44). Total, and some individual, fatty acid concentrations were greater in severe COPD than CON (FDR<0.05; n=16; d=0.56-1.02). After

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adjusting for FEV_1%predicted, we observed that grouped diacylglycerides (ρ=-0.745; FDR=0.03) and triacylglycerides (ρ=-0.811; FDR=0.01) were negatively associated with muscle oxidative capacity, but not physical activity, in severe COPD (n=14). **Conclusion**: Strong negative associations relate impaired mitochondrial function to the accumulation of serum aclyglycerides in severe COPD.

Key Words: metabolomics, mitochondria, physical activity, sphingomyelin, fatty acid

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is associated with airway inflammation, mucus hypersecretion, and pulmonary emphysema; each contributing to expiratory flow limitation. Unifying symptoms of these heterogeneous phenotypes are dyspnea on exertion and exercise intolerance. Exercise intolerance and physical inactivity, not pulmonary obstruction, are the strongest predictors of mortality in COPD (1). Although no therapies beyond smoking cessation are yet proven to slow disease progression or reduce mortality, pulmonary rehabilitation - a multidisciplinary program that includes exercise training - is the most effective treatment in releveling symptoms, increasing quality of life, reducing hospitalizations and morbidity in COPD patients (2). A primary benefit of pulmonary rehabilitation in COPD is symptom relief and increased exercise tolerance, which are mediated by ameliorating skeletal muscle deficits in oxidative capacity, thereby delaying the onset of exercise-induced metabolic acidosis and reducing the ventilatory demands for a given activity (3).

Several studies of serum metabolomics show metabolic dysregulation in COPD (4-6). Alterations in sphingolipid metabolism are common in COPD, suggesting a deficit in lipid metabolism that may contribute to smoking-induced lung damage through mitophagy-mediated necroptosis (7). Altered mitochondrial β-oxidation, tryptophan metabolism, carnitine/acylcarnitine, reduced polyunsaturated fatty acids and high oxidative stress are common findings following cigarette smoke exposure and in COPD metabolomic analyses (8). Furthermore, cigarette smoke exposure

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is associated with the accumulation of cytotoxic ceramides in lung epithelial cells and reduced mitochondrial respiration in skeletal muscle, resulting in insulin resistance and poor glucose tolerance (7, 9).

Muscle deconditioning following physical inactivity is associated with a reduced fraction of whole-body ATP turnover that is derived from mitochondrial β-oxidation during rest or exercise (10). Loss of mitochondrial oxidative capacity in skeletal muscle is, therefore, a primary variable implicated in mediating the association between hyperlipidemia and COPD. As both physical activity and oxidative capacity are negatively associated with COPD severity (11), this study aimed to determine whether lipid metabolite dysregulation in COPD was associated with muscle oxidative capacity and/or physical inactivity. We hypothesized that alteration of lipid metabolites in COPD would be associated with muscle oxidative capacity, independent of pulmonary function.

METHODS

Study population

The population was drawn from the single-center Muscle Health Study, an ancillary study of COPDGene (ClinicalsTrials.gov Identifier NCT00608764). A total of 245 current or former smokers participated in the Muscle Health Study at The Lundquist Institute between 2013 and 2016. Inclusion and exclusion criteria were determined by the COPDGene study design (12). Participants were non-Hispanic White or African American, aged 45–80 years, and all had ≥10

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pack-year smoking history. In addition, those with known or suspected cancer or recent (within 3 months) hospitalization were excluded. Of the 245 subjects, 107 had serum samples collected for metabolomic investigation: 44 with COPD and 63 with normal spirometry acted as controls (CON). Participants gave written informed consent to participate as approved by the Institutional Review Board at The Lundquist Institute. Data of muscle oxidative capacity and pulmonary function from these participants has been previously reported (13).

Additional methodological details are provided in Supplemental Digital Content (see Supplemental Digital Content 1, methods for clinical assessments, muscle oxidative capacity, prime metabolomics and lipidomics, http://links.lww.com/MSS/C49).

Clinical assessments

As part of the COPDGene study protocol, clinical data collected included demographics, vital signs, medical and smoking history, and current medications. Spirometry was performed according to American Thoracic Society guidelines (14). Lung diffusing capacity for carbon monoxide (DL_{CO}) was measured after post-bronchodilator spirometry assessment (15). Resting arterial oxygen saturation was measured using pulse oximetry (SpO_2).

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Muscle oxidative capacity

Oxidative capacity of the medial *gastrocnemius* muscle (*k*) was assessed using near-infrared spectroscopy (NIRS) as described previously (16). Prior work demonstrates, using direct measurements in single muscle fibers of varied biochemical phenotypes, that *k* is directly proportional to muscle oxidative capacity (17). The average *k* of two repeat measurements is reported.

Physical activity

At the end of the visit, participants received a triaxial accelerometer (DynaPort MoveMonitor, McRoberts BV, The Hague, the Netherlands) to assess number of steps per day and physical activity reported as vector magnitude units (VMU)/min. Activity measurements were considered complete if the participant maintained at least 15 hours of wearing time per day for at least 5 of the 7 days.

Prime metabolomics and lipidomics

Blood was collected from a peripheral vein using a serum separator tube (8.5 mL, BD Vacutainer) and the serum aliquoted (1 mL) and stored at -80°C for subsequent analysis. Blood was collected typically ~3-4 hours after taking a usual breakfast. Serum samples were shipped to West Coast Metabolomics Center at the University of California for metabolomic analysis.

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Statistics

For general statistics, data are presented as mean ± SEM. Baseline subject characteristics, muscle oxidative capacity and physical activity were compared by ANOVA and Dunnett's *post hoc* test using CON as the reference group (continuous variables) or chi^2-test (categorical variables). Routine metabolomics data analysis was performed with MetaboAnlyst 3.0 (www.metaboanalyst.ca). Differences in metabolite concentrations among groups were assessed by analysis of variance (ANOVA) and Fisher's LSD *post hoc* test accounting for multiple comparisons using false discovery rate (FDR). Association between muscle oxidative capacity or physical activity and metabolite concentration was initially assessed using Spearman correlation stratified for GOLD class. Subsequently, lipid metabolites were categorized into 17 metabolite classes, grouped by their chemical properties, and partial correlation performed with adjustment for FEV_1 %predicted.

All comparisons were two-sided. Effect sizes are reported as Cohen's d (d). For metabolite analyses, FDR ≤ 0.05 was considered statistically significant. For other analyses, $P \leq 0.05$ was considered statistically significant.

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RESULTS

Participant demographics and clinical characteristics

The baseline characteristics of the study participants are presented in **Table 1**. Overall, 55% of the 107 participants were female, 52% were African American and 48% were non-Hispanic White. COPD patients were significantly older than CON, less likely to be current smokers, and had a greater representation of non-Hispanic White participants. There were no significant differences between the groups in sex, weight, BMI, smoking history, diabetes or hypertension. By definition, FEV_1/FVC and FEV_1 %predicted were lower in COPD than CON. DL_{CO} was significantly lower in COPD than CON, but there was no difference in resting SpO_2 between the groups (**Table 1**). Additional analyses were made on a sub-group comprised of only severe COPD (FEV_1 < 50% predicted, n=16). This sub-population is shown separately in **Table 1**. Except for the degree of pulmonary obstruction (by definition) and a lower SpO_2 than CON (not clinically significant: 97.8±2.4 vs 96.1±0.7 %; d = 0.67), severe COPD patients had baseline characteristics that were similar to the whole COPD group (**Table 1**).

Muscle oxidative capacity and physical activity

Non-invasive measurement of the $m\dot{V}O_2$ recovery rate constant, *k*, was successful in 42 (95%) COPD and 56 (89%) CON participants. *k* was significantly lower in COPD than CON (1.32±0.07 min^{-1} vs. 1.68±0.06 min^{-1}; $P < 0.0001$; d = 0.81, **Figure 1A**), and lower still in the COPD patents with severe disease (FEV_1 <50 %predicted) (1.12 ±0.05 min^{-1}; $P < 0.0001$ vs CON; n = 14; d =

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1.58) (**Figure 1A**). Forty-two (95%) COPD and 56 (89%) CON completed at least 5 days of triaxial accelerometer monitoring as designed (≥15 hours per day). Daily number of steps was not different between COPD and CON (5254±701 vs. 6188±442 steps/day, $P = 0.375$; d = 0.23) but was lower in severe COPD (3171±568 steps/day; $P = 0.010$ vs. CON; d = 1.04) (**Figure 1B**). Physical activity was not different between COPD and CON (353±43 vs. 450±50 VMU/min; $P = 0.233$; d = 0.30) but was lower in severe COPD (170±26 VMU/min; $P = 0.004$ vs. CON; d = 1.04) (**Figure 1C**).

Sex differences in serum sphingomyelin were diminished in COPD patients

Lipidomics analysis using sex as a covariate revealed 24 differentially expressed lipids between all COPD and CON (one-way ANOVA) (**Figure 2A**; FDR < 0.05; d = 0.36-1.31), of which 19 were sphingomyelins. *Post hoc* analysis showed that this effect was driven by a significant difference between males and females in the CON group (see Table, Supplemental Digital Content 2, list of metabolites and differences, http://links.lww.com/MSS/C50). In CON, sphingomyelin concentrations were generally greater in females than males, and 38 sphingomyelin species were identified significantly greater in females than in males (**Figure 2B**; FDR < 0.05; d = 0.58-1.32; see Table, Supplemental Digital Content 3, list of sphingomyelins that were significantly different between males and females in CON, http://links.lww.com/MSS/C51). Conversely, in COPD, only 4 sphingomyelins were significantly greater in females than males (**Figure 2C**; FDR < 0.05; d = 1.00-1.35; see Table, Supplemental Digital Content 4, list of sphingomyelins that were

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significantly different between males and females in COPD, http://links.lww.com/MSS/C52). These data indicate that the anticipated differences in sphingomyelin concentrations between the sexes were diminished in COPD patients.

Fatty acid metabolites were increased in severe COPD patients

Prime metabolomics and lipidomics analysis identified 129 prime metabolites and 470 lipids with known identity in the serum of study participants. Metabolite concentrations were not significantly different between COPD and CON. However, several metabolites, predominantly fatty acids, were differentially expressed in severe COPD (FEV_1 < 50 %predicted; n = 16) compared with CON. In lipidomics analysis, total fatty acid concentration was significantly greater in severe COPD than in CON (**Figure 3A**; $P < 0.05$; d = 1.02). This was predominantly due to 4 fatty acids that were significantly greater in severe COPD than in CON (**Figure 3B**; FDR < 0.05; d = 0.83-0.89). In the prime metabolites, the concentrations of 7 fatty acids were significantly greater in severe COPD than in CON (**Figure 3C**; FDR < 0.05; d = 0.59-1.02).

Acylglycerides were negatively associated with muscle oxidative capacity in severe COPD

Spearman correlation analysis was employed to identify whether metabolite concentrations were associated with the $m\dot{V}O_2$ recovery rate constant (*k*) and/or physical activity. All individual diacylglyceride (DG) and triacylglyceride (TG) metabolites had negative correlation with muscle oxidative capacity after adjusting for FEV_1 %predicted (which incorporates adjustment for age,

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sex, race and height (18)). There were no significant associations between TG or DG with age, BMI, resting systolic or diastolic blood pressure, current smoking status, smoking history, FEV_1 %predicted, incidence of diabetes or hypertension, steps/day or VMU/min. Overall, 7 out of 8 DG and 48 out of 102 TG were nominally negatively associated with muscle oxidative capacity in severe COPD (**Figure 4A**, $P < 0.05$; n = 14).

Next, lipids were grouped into 17 classes based on their characteristics, and partial correlations were re-assessed. Following adjustment for FEV_1 %predicted and correcting for FDR, we found that muscle oxidative capacity was negatively correlated with diacylglyceride concentration (ρ = -0.7447; FDR = 0.03) and triacylglyceride concentration (ρ = -0.8118; FDR = 0.01) in severe COPD patients (n = 14), but not in CON (n = 56). Neither daily steps nor physical activity were significantly associated in partial correlation with the concentrations of any metabolite group (**Figure 4B**). Adjustment of the partial correlation analysis using DL_{CO} (a slightly stronger correlate of grouped metabolites than FEV_1 %predicted), did not change the significant correlation between *k* and DG (ρ = -0.7544; FDR = 0.02) or TG (ρ = -0.8116; FDR = 0.01) in the severe COPD group (n = 14). Although there was no significant association between BMI and DG or TG, we also sought to adjust for BMI due to its potential association with hyperlipidemia. This adjustment did not substantively affect the correlation between *k* and TG (ρ = -0.7579; FDR = 0.04), although the correlation was weakened between *k* and DG (ρ = -0.6746; FDR = 0.09). There

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remained no association between any lipid metabolite group and any measure of physical activity after adjustment for covariates.

DISCUSSION

In this study, we conducted both prime metabolomic and lipidomics analyses in COPD patients and controls, to identify whether serum metabolites were associated with physical activity and/or muscle mitochondrial oxidative capacity. We observed: 1) 24 lipids, including 19 sphingomyelins, were differentially expressed in COPD with sex as covariant; 2) sex-dependent differences in sphingomyelin concentration in controls were diminished in COPD patients; 3) severe COPD patients (n = 16) had elevated serum total fatty acids, centered on 8 individual fatty acid metabolites; and, 4) serum concentrations of di- and tri-acylglycerides were negatively associated with muscle oxidative capacity, and not physical activity, in severe COPD (n = 14). Previous metabolomics studies of spirometrically-defined COPD reported dysregulation in several serum metabolite classes (4-6). Here we identify that skeletal muscle deconditioning in the form of reduced muscle oxidative capacity, common in COPD, may underlie metabolic dysregulation of di- and tri-acylglycerides in patients with severe pulmonary obstruction.

Dysregulation of sphingolipid metabolism is common in patients with COPD. In an untargeted lipidomic analysis of sputum samples, Telenga *et al*. (19) demonstrated that sphingolipids, including several serum sphingomyelins, were significantly greater in smokers with COPD than

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those without COPD. Thirteen individual serum lipid metabolites, including one sphingomyelin, showed strong negative association with FEV_1 and inflammation in sputum. Telenga *et al*. also found that two months of smoking cessation reduced concentration of 26 sphingomyelins in both groups (19). Others have demonstrated a significant negative association between sphingomyelin metabolites and emphysema from chest CT measurements (5) or COPD exacerbation severity (7).

Consistent with studies of healthy subjects (20), our data showed greater serum sphingomyelin concentration in females than in males in our control group, which consisted of current or former smokers with normal spirometry. We found that this sex difference was diminished in all COPD patients, suggesting a sex-dependent alteration of sphingomyelin metabolism in COPD. Intracellular ceramide concentration is regulated by sphingolipid metabolism and is implicated in cigarette smoking induced mitophagy (7). Sphingolipid metabolism was also associated with emphysema progression in sub-phenotyping analysis (21). Whether the diminishing sex-differences in circulating sphingomyelin metabolism underlie the more rapid progression of COPD observed in women than in men deserves further attention.

We identified that the serum concentration of total fatty acids, and some individual fatty acids, were significantly greater in severe COPD (n = 16) than in controls (n = 63). This observation was in contrast with a small study of COPD (including 10 patients with severe COPD) by Wada *et al.*

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where total free fatty acid concentration was significantly lower in COPD than in healthy controls (22). This discrepancy may reflect the disease stage of the subjects in each study; BMI in the severe COPD patients in the study of Wada *et al*. was significantly lower than controls, while there was no difference in BMI between groups in our study.

The role of individual circulating fatty acids in the progression of pulmonary, cardiovascular or metabolic disease in COPD patients is not well studied. For example, increased dietary intake of fatty acids is associated with greater expiratory flow limitation in COPD patients, while dietary intake of pentadecylic acid may improve lung function in these patients (23). We found 7 individual fatty acids in prime analysis and 4 in lipidomics that were greater in severe COPD, with 3 individual fatty acids recapitulated in both analytic approaches (myristic acid, plamitoleic acid and heptadecanoic acid). Myristic acid potentiates palmitic acid-induced lipotoxicity, likely through mitochondria-related mechanisms (24). Similar to a previous investigation (25), we found that the monounsaturated fatty acid, palmitoleic acid, was greater in severe COPD; which is associated with greater high and low density lipoprotein cholesterol (26). On the other hand, lauric acid was also increased in severe COPD in our prime analysis, which is implicated in potentially beneficial effects on cholesterol, insulin resistance and inflammation. Given the low mitochondrial oxidative capacity we found in muscles of severe COPD patients (13), and the known greater odds of cardiometabolic disease in severe COPD, the differential effects on COPD or COPD progression of the individual fatty acids identified here deserve further study.

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Despite variability in the prevalence of hyperlipidemia, subclinical atherosclerosis occurs at a greater than expected prevalence in COPD, and is associated with more frequent exacerbations (27). Regular physical activity and increased mitochondrial function are associated with lower blood lipids and triglycerides, and are protective of metabolic and cardiovascular disease (28). Therefore, identifying whether differences in physical activity and/or mitochondrial function underlie the observations of lipid metabolite dysregulation in COPD was a major thrust of this study. Overall, we did not find significant associations between lipid metabolites and either muscle oxidative capacity or physical activity when considering differences between all COPD patients and controls. However, there was a significant ceiling effect on these variables, and so we focused our analyses on severe disease (FEV_1 < 50 %predicted). In severe COPD, there was a strong negative association between muscle oxidative capacity and serum di- or tri-acylglycerides (ρ ranged -0.75 to -0.81; n = 14). These associations remained even after adjusting for false discovery rate, and FEV_1 or DL_{CO} or BMI. It was striking that physical activity (either steps/day or VMU/min; n = 16) was not significantly associated any serum lipid metabolite or metabolite group investigated. This distinction is important because it implies that mitochondrial metabolic health, rather than physical activity *per se*, may be involved in lipid dysregulation in severe COPD.

Support for this concept is found elsewhere in biology with, for example: a) no reduction in mortality in mice selectively bred for high lifelong energy expenditure, whereas rats selectively bred for endurance running capacity begets high muscle oxidative capacity and a ~40% increase in

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median lifespan (29); b) while high rates of physical activity are known to reduce all-cause mortality risk (30), the hazard ratio for mortality in 8,171 male veterans was reduced by ~50% when stratifying by exercise capacity compared with stratifying for physical activity (31); c) there was no survival benefit of increasing self-reported physical activity in longitudinal study of 1,270 COPD patients with a median follow-up duration of 17 years (32). On the other hand, it is well established that exercise training, as part of a pulmonary rehabilitation program, increases muscle oxidative capacity (33), reduces 1-year hospital readmission (odds ratio vs. usual care = 0.44 (95% confidence interval 0.21 – 0.91), and potentially reduces 1-year mortality (odd ratio = 0.68 (0.28 – 1.67)) (2), without an impact on physical activity (34); d) changes in fat free mass and exercise capacity (but not physical activity) in COPD are also associated with rapid decline in health status (35).

Metabolic syndrome is prevalent in COPD (36). Previous findings identified that an increase in circulating triglycerides is a major risk factor for 5-year mortality in COPD patients (37). Hypertriglyceridemia and systemic inflammation are independent predictors of elevated plasminogen activator inhibitor-1 in COPD, a major inhibitor of fibrinolysis, associated with thrombosis, obesity, insulin resistance, dyslipidemia, and premature aging; each prevalent in COPD (38). Intracellular accumulation of triglycerides and other fatty acids, promote endoplasmic reticulum stress, mitochondrial uncoupling and oxidative stress, which terminates in inflammation and cell death (39). Perivascular adipose accumulation seems to trigger atherosclerosis and

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hypertension, also prevalent in COPD. The association between circulating triglycerides and muscle mitochondrial oxidative capacity we identified in severe COPD provides a strong justification for the role of increasing physical fitness in reducing cardiovascular and metabolic risk in this patient population. Our proposal is that attempts to redress lipid metabolic deficits in COPD should not focus on simply diet or activity interventions, but specifically on obtaining the health-related benefits associated with increasing muscle (and other tissue) mitochondrial oxidative capacity.

There are several limitations to this study. The number of subjects is low, particularly in the severe COPD group, which limited the statistical power to detect associations between individual lipid metabolites and muscle oxidative capacity or physical activity. Diet and circadian rhythm are known to regulate metabolism. Our serum samples were not collected with dietary control or at the same circadian time range, both of which could influence postprandial lipid profile and contribute to variation in metabolite concentrations. In addition, increased carbohydrate and fatty acid intake are associated with worse pulmonary function (23). In attempt to mitigate this potential confounder, our findings remained after adjusting for FEV_1 %predicted. We were not able to include measurements of adiposity or analysis of systemic markers of inflammation, which could have contributed to our understanding of lipid dysregulation. The measure of muscle oxidative capacity we used is non-invasive; nevertheless, it was successful in 92% of participants

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and we have demonstrated this method has strong reproducibility in COPD patients (16), while others have shown good association (r = 0.61-0.74) with muscle biopsy (40).

In conclusion, we observed that 24 lipids, including 19 sphingomyelins, were increased in COPD with sex as covariant, and that sex-dependent differences in sphingomyelin concentration in controls were diminished in COPD patients. We also found that severe COPD patients had elevated serum total fatty acids, which centered on 8 individual fatty acid metabolites. These findings may in part underlie the more rapid progression of COPD observed in women than in men and the high prevalence of cardiovascular disease in COPD patients. Lipid dysregulation that was negatively associated with muscle oxidative capacity (ρ ranged -0.75 to -0.81; n = 14), and not physical activity (n = 16); a negative association which remained despite adjustment for FEV_1 %predicted, DL_{CO} or BMI. The strong negative association we identified between di- or tri-acylglycerides and muscle oxidative capacity, suggests that impaired mitochondrial function may play a role in the accumulation of serum aclyglycerides in severe COPD, and provides a strong rationale for targeting mitochondrial deficits by exercise training, or other means, to improve outcomes in this patient population.

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CONFLICT OF INTEREST

The authors declare no conflict of interest. The authors declare that the results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of this study do not constitute endorsement by ACSM.

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FIGURE LEGENDS

Figure 1. Muscle oxidative capacity and physical activity is reduced in severe COPD patients compared with controls (CON). A) Muscle oxygen consumption recovery rate constant (*k*, min^{-1}), which is linearly proportional to muscle oxidative capacity (CON n=56; ALL COPD n=42; severe COPD n=14). **B**) Daily steps (CON n=56; ALL COPD n=42; severe COPD n=16). **C**) Average daily VMU/min (CON n=56; ALL COPD n=42; severe COPD n=16).

Figure 2. The sex difference of serum sphingomyelin concentration was diminished in COPD patients compared with controls (CON). (A) ANOVA of lipidomics of COPD patients (n=44) and CON (n=63) with sex as a covariant. Filled red circles indicate metabolites with significant difference among groups. **(B)** Comparison of sphingomyelin (SM) concentration between male and female CON subjects (n=63). **(C)** Comparison of sphingomyelin (SM) concentration between male and female COPD patients (n=44). Filled pink circles indicate metabolites with significant difference between the sexes. Data were corrected for false discovery rate (FDR).

Figure 3. Fatty acids were increased in severe COPD patients compared with controls. Lipid metabolites in severe COPD patients (FEV_1 < 50% predicted, open symbols/open bars) compared with CON (filled symbols/filled bars). **(A)** Total fatty acids were significantly greater in severe COPD patients compared with CON in lipidomics analysis. **(B)** Four fatty individual acids were

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identified as significantly greater in severe COPD patients in lipidomics analysis. **(C)** Seven fatty acids were identified as significantly greater in severe COPD patients in prime metabolite analysis. Data were corrected for false discovery rate (FDR): * FDR<0.05; ** FDR<0.01; *** FDR< 0.005; **** FDR<0.001; CON n=63; severe COPD n=16.

Figure 4. Diacylglyceride (DG) and triacylglyceride (TG) classes of lipid metabolites were correlated with muscle oxidative capacity in severe COPD. (A) Spearman correlation analysis of 470 individual lipid metabolites with muscle oxidative capacity in severe COPD. Individual metabolites were placed into classes based on their characteristics, shown in panel B. **(B)** Partial correlation of grouped lipid metabolites with muscle oxidative capacity, daily steps and physical activity (VMU/min). Data were adjusted for FEV_1 % predicated and corrected for FDR. Statistically significant associations were identified for DG and TG classes (panel B). DG and TG regions within the individual metabolite data are highlighted in panel A by horizontal dash. * FDR<0.05. Severe COPD n=14-16.

LIST OF SUPPLEMENTAL DIGITAL CONTENT

1. Supplemental methods
2. Supplemental Table 1
3. Supplemental Table 2
4. Supplemental Table 3

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Fig. 1

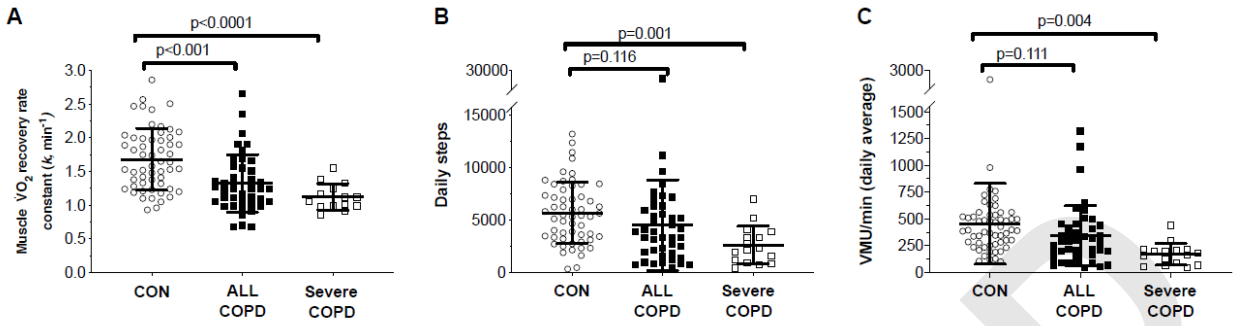

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Figure 2

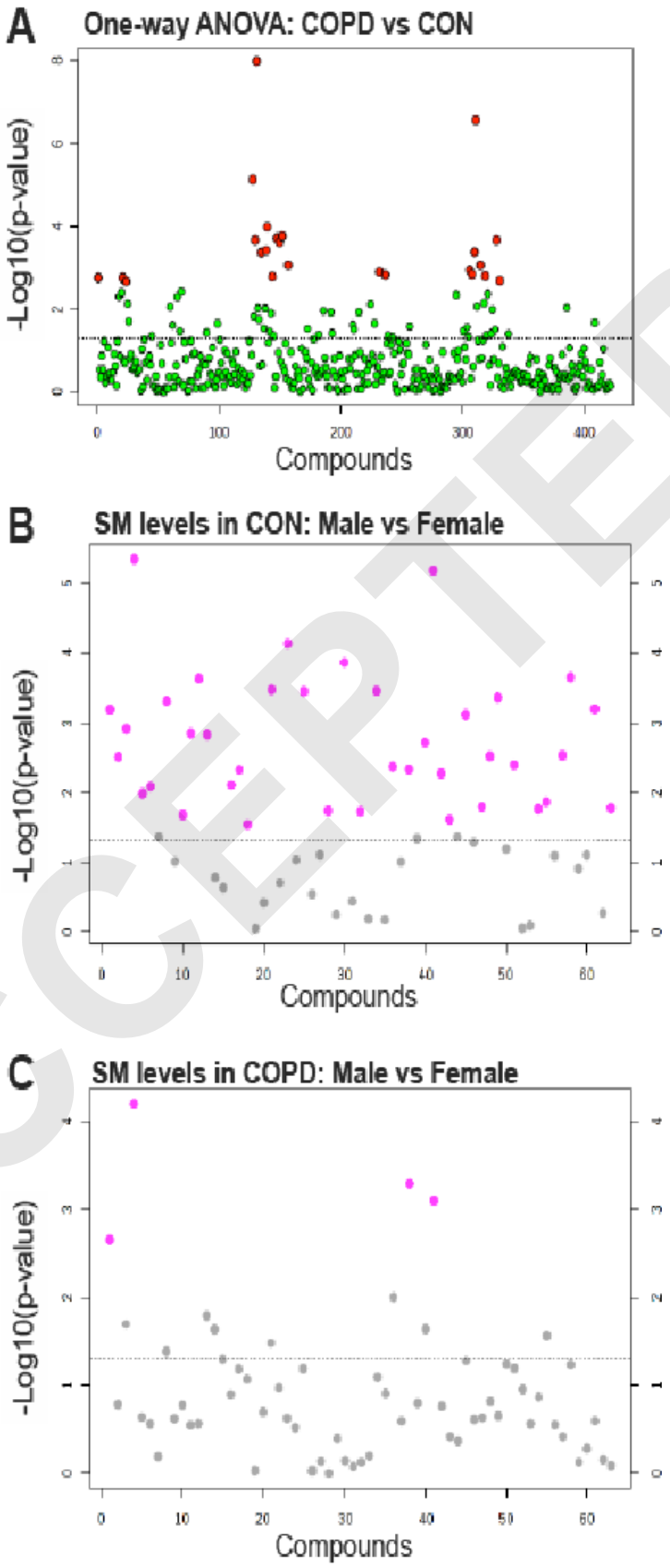

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Figure 3

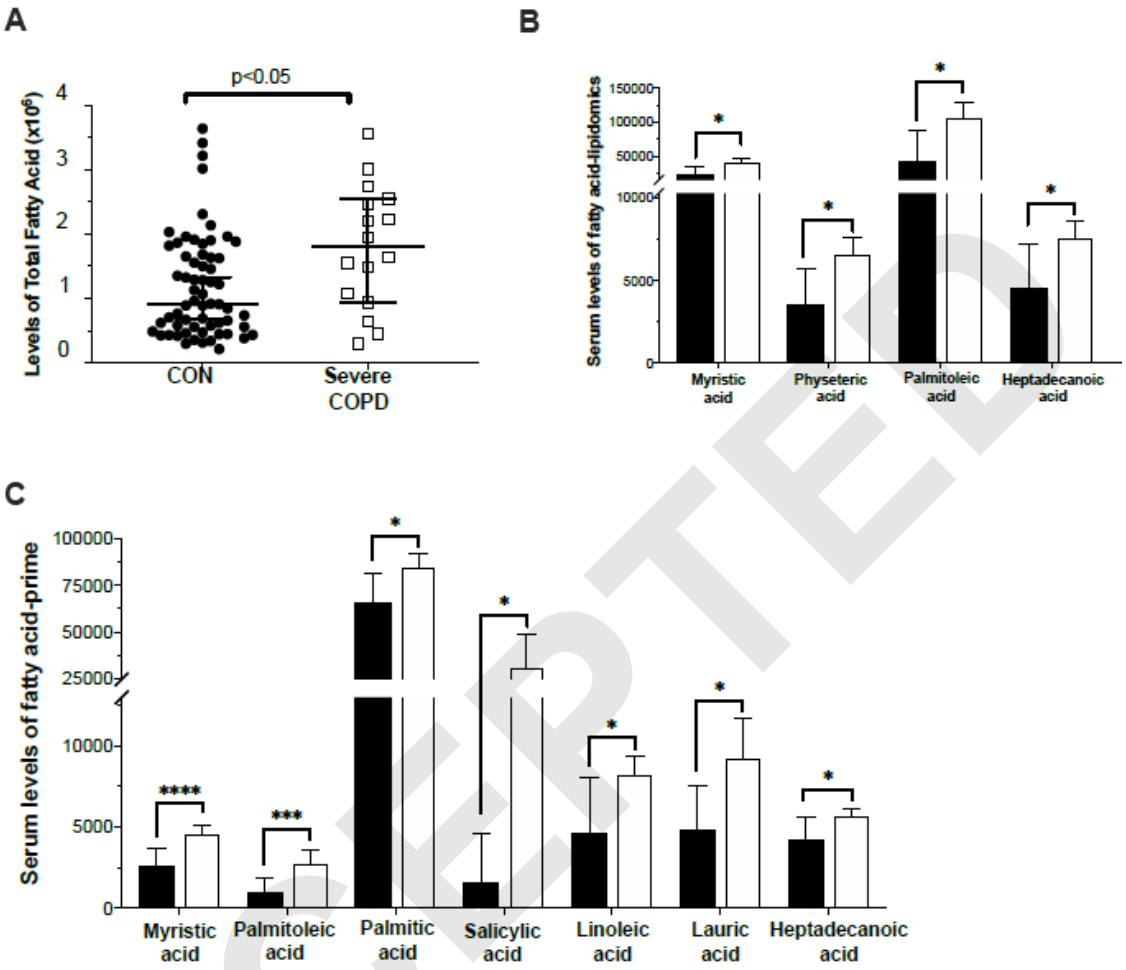

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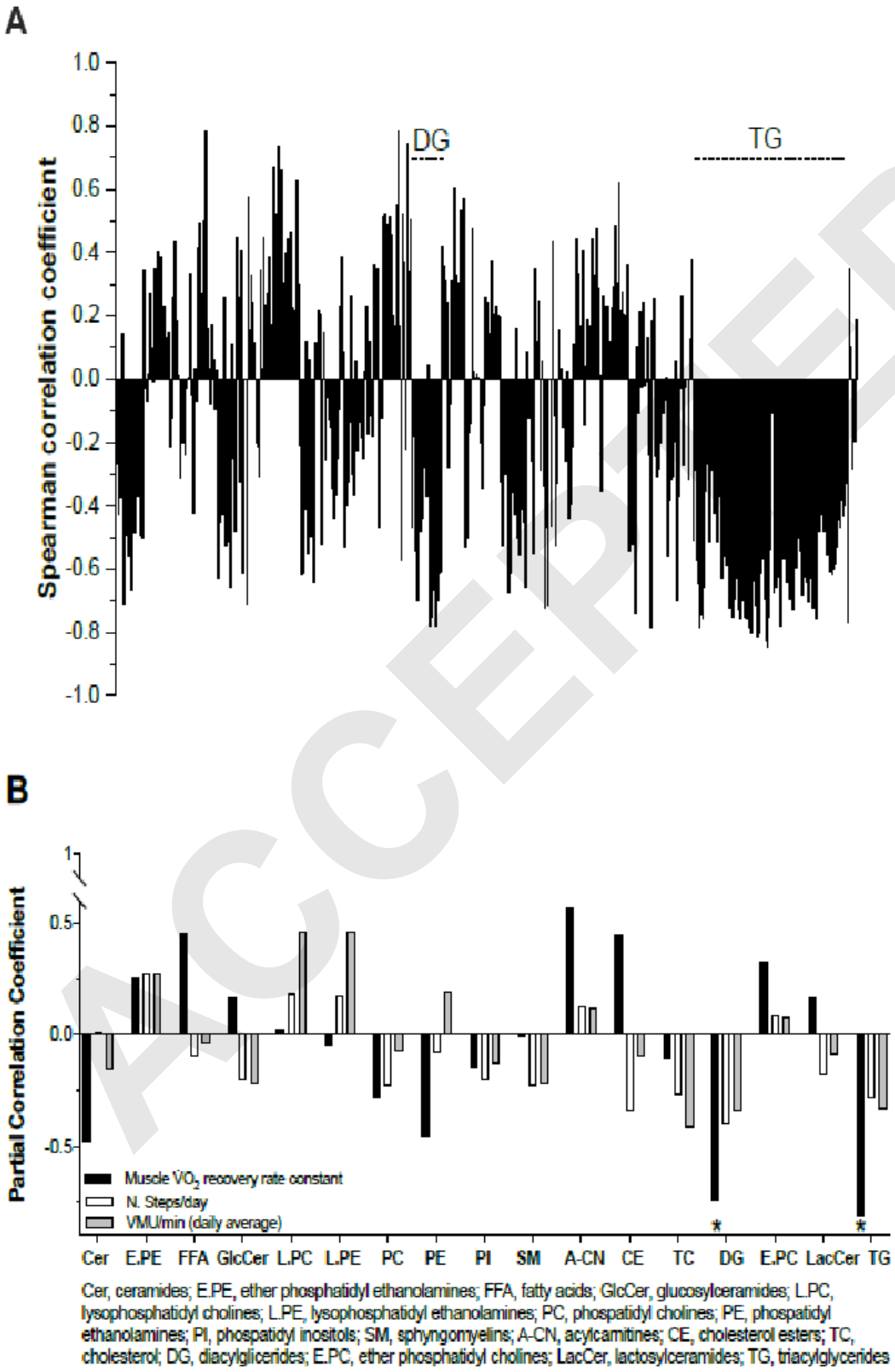
Figure 4



Cer, ceramides; E.PE, ether phosphatidyl ethanolamines; FFA, fatty acids; GlcCer, glucosylceramides; L.PC, lysophosphatidyl cholines; L.PE, lysophosphatidyl ethanolamines; PC, phospatidyl cholines; PE, phospatidyl ethanolamines; PI, phospatidyl inositols; SM, sphyngomyelins; A-CN, acylcamitines; CE, cholesterol esters; TC, cholesterol; DG, diacylglicerides; E.PC, ether phosphatidyl cholines; LacCer, lactosylceramides; TG, triacylglycerides

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Table 1. Participant characteristics.

	Unit	CON	ALL COPD	Severe COPD	p value ALL COPD vs CON	p value Severe COPD vs CON
Number of Subjects	n	63	44	16	-	-
GOLD 1/2/3/4	n	0 / 0 / 0 / 0	14 / 14 / 9 / 7	9 / 7	-	-
Age	years	61.2 ± 1.3	65.6 ± 1.4	66.6 ± 1.6	0.039	0.086
Sex, M / F	n	29 / 34	21 / 23	6 / 10	0.981	0.786
Race, NHW / AA	n	21 / 42	30 / 14	13 / 3	<0.0001	<0.001
Weight	kg	85.3 ± 2.7	79.2 ± 2.6	78.1 ± 4.7	0.209	0.330
BMI	kg/m^2	29.8 ± 0.9	28.2 ± 0.9	29.2 ± 1.8	0.405	0.941
Smoking history	pack-years	39.2 ± 2. 6	46.5 ± 3.6	47.0 ± 6.4	0.187	0.372
Smoking duration	years	35.6 ± 1.3	37.2 ± 1.5	35.4 ± 2.7	0.656	0.997
Current smoker	n (%)	34 (54)	13 (30)	3 (13)	0.020	0.018
FEV_1/FVC	%	79.6 ± 0.7	52.5 ± 2.4	35.8 ± 3.3	<0.0001	<0.0001
FEV_1 % predicted	%	93.4 ± 2.2	61.4 ± 4.1	31.6 ± 2.9	<0.0001	<0.0001
DL_{CO}	mL/min/mmHg	75.9 ± 2.2	61.3 ± 3.6	41.8 ± 3.9	0.001	<0.0001

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Diabetes	n (%)	13 (21)	4 (9)	1 (6)	0.181	0.265
Hypertension	n (%)	36 (57)	21 (48)	8 (50)	0.557	0.844
SpO_2	%	97.8 ± 2.4	97.3 ± 2.0	96.1 ± 0.7	0.421	0.015

GOLD, global initiative for obstructive lung disease spirometric classification (1, Mild; 2, Moderate; 3, Severe; 4, Very-severe); NHW, non-Hispanic White; AA, African American; BMI, body mass index; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; DL_{CO}, diffusing capacity for carbon monoxide; SpO_2, oxyhemoglobin saturation by pulse oximetry. Spirometric variables are post-bronchodilator values.

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Serum Acylglycerols Inversely Associate with Muscle Oxidative Capacity in Severe COPD

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SUPPLEMENTAL METHODS

Clinical assessments

As part of the COPDGene study protocol, clinical data collected included demographics, vital signs, medical and smoking history, and current medications.

Spirometry was performed according to American Thoracic Society guidelines (21) using a dual beam Doppler ultrasound-based spirometer (EasyOne Pro, Ndd Medical, Zürich, Switzerland). Participants inhaled two puffs of metered dose albuterol sulfate (ProAir HFA, Teva Respiratory, Horsham, PA, USA) 15 minutes before spirometric testing. Forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) were measured from the greatest FEV_1 and FVC from up to eight maximum expiratory maneuvers, where the greatest two measurements were within 150 mL.

Lung diffusing capacity for carbon monoxide (DL_{CO}) was measured after the post-bronchodilator spirometry assessment (EasyOne Pro DL_{CO}, Ndd Medical, Zürich, Switzerland) (22). DL_{CO} measurement was made following an exhalation to residual volume, followed by a rapid inspiration to total lung capacity and breath hold for 8-12 seconds. The maneuver ended with a complete exhalation to residual volume and resumption of normal breathing. This procedure was performed at least 2 and no more than 5 times. The test was accepted when 2 maneuvers were within 3 $mL.min^{-1}.mmHg$ or within 10% of the greatest value.

Resting arterial oxygen saturation was measured using pulse oximetry (SpO_2; Rad-5 Pulse Oximeter MasimoSET®, Masimo Co., Irvine, CA).

Muscle oxidative capacity

Oxidative capacity of the medial *gastrocnemius* muscle was assessed using near-infrared spectroscopy (NIRS) (23, 24). Briefly, a wireless, portable, continuous-wave, spatially-resolved NIRS probe (PortaMon, Artinis, The Netherlands) was wrapped in plastic film and secured with an elastic bandage to the belly of the medial *gastrocnemius* to measure relative change in oxygenated and deoxygenated myoglobin + hemoglobin. From these, tissue saturation index (TSI, %), and the relative change in total tissue myoglobin + hemoglobin was calculated. A 13 x 85 cm rapid-inflation pressure-cuff (SC12D, Hokanson, USA) was placed proximally on the thigh of the same leg and attached to a rapid cuff-inflator (E20, Hokanson, USA). Participants were

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familiarized with rapid cuff inflation procedures. During familiarization the pressure required for arterial occlusion was identified (range 230–300 mmHg). Participants lay at rest for ~3 min to determine resting muscle TSI and SpO_2 at the fingertip (Rad-5 Pulse Oximeter MasimoSET®, Masimo Co., Irvine, CA). Participants then performed 10–12 cycles of ~1 Hz plantar-flexion muscle contractions, followed immediately by arterial occlusion until a stable minimum TSI was reached, or for 5 min (whichever came first). This was used to establish an individualized physiologic range (maximum and minimum) of muscle TSI(23) After at least 3 min recovery, participants performed ~10-15 s plantar-flexion muscle contractions to increase muscle oxygen uptake ($m\dot{V}O_2$) and desaturate the muscle to ~50% of physiologic range(23) ; this was followed immediately by a series of intermittent arterial occlusions (5 occlusions for 5 s; 10 for 10 s; each separated by 5–20 s recovery; total duration ~6 min). This last ~6 min phase was repeated after ~2 min rest. For each brief arterial occlusion, the rate of decline in TSI ($\%.s^{-1}$) was calculated to determine relative $m\dot{V}O_2$. The $m\dot{V}O_2$ recovery rate constant (k, min^{-1}) was measured by non-linear least-squares regression of the $m\dot{V}O_2$ exponential recovery (OriginPro v8.6, OriginLab Co., Northampton, USA) (23). k is directly proportional to muscle oxidative capacity (25). The average k of two repeats is reported.

Prime metabolomics and lipidomics

Post-prandial blood was collected from a peripheral vein using a serum separator tube (8.5 mL, BD Vacutainer) and the serum aliquoted (1 mL) and stored at -80°C for subsequent analysis.

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Serum samples were shipped to West Coast Metabolomics Center at the University of California, Davis on dry ice for metabolomics analysis. For untargeted assessment of primary metabolites, gas chromatography time-of-flight mass spectrometry (GC-TOF MS) method was used as described (26). Briefly, 30 µL serum samples and internal standards were extracted and derivatized by silylation/methyloximation. Metabolites were separated using an Agilent 6809 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA). Mass spectrometry was performed with a Leco Pegasus IV time of flight mass spectrometer. Peak heights of quantifier ions for each metabolite were determined by comparing with internal standards. For untargeted lipidomics, serum samples were extracted in methyl tert-butyl ether with the addition of internal standards, followed by ultra-high pressure liquid chromatography on a Waters CSH column, interfaced to a quadrupole time-of-flight mass spectrometer (high resolution, accurate mass), with a 15-minute total run time. Peak areas of lipid species within the range of the calibration curves were analyzed by comparing the individual peak areas with those of corresponding internal standards for determining the final concentration of each metabolite. Data were collected in both positive and negative ion mode and analyzed using MassHunter (Agilent).

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Supplementary Table SDC 1. Lipid metabolites that showed sex differences in one-way ANOVA. Nineteen out of 24 metabolites that were different between the sexes were sphingomyelins.

Metabolite	F value	P value	FDR	Fisher's LSD
SM d322 A	16.224	1.04E-08	4.39E-06	COPD-F vs. COPD-M; COPD-F vs. CON-M; CON-F vs. COPD-M; CON-F vs.CON-M
SM d322 B	13.068	2.68E-07	5.66E-05	COPD-F vs. COPD-M; COPD-F vs.CON-M; CON-F vs. COPD-M; CON-F vs.CON-M
SM d301A	10.025	7.45E-06	0.001048	COPD-F vs. COPD-M; COPD-F vs.CON-F; COPD-F vs.CON-M; Con-F vs.CON-M
SM d371 A	7.7381	0.000104	0.010925	COPD-F vs. COPD-M; COPD-F vs.CON-M; CON-F vs.CON-M
SM d412 A	7.2925	0.000175	0.011198	COPD-F vs.CON-M; COPD-M vs.CON-M; CON-F vs.CON-M
SM d402 A	7.2063	0.000194	0.011198	COPD-F vs. COPD-M; COPD-F vs.CON-M; CON-F vs.CON-M
SM d321 A	7.1344	0.000212	0.011198	COPD-Fvs. COPD-M; COPD-Fvs.CON-M; CON-F vs.CON-M
SM d412 B	7.1313	0.000212	0.011198	COPD-F vs.CON-M; CON-F vs.CON-M

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SM d403	7.0219	0.000242	0.011338	COPD-F vs.CON-M; CON-F vs.COPD-M; CON-F vs.CON-M
SM d363 A	6.6337	0.000385	0.015247	COPD-F vs.CON-M; COPD-M vs.CON-M; CON-F vs.CON-M
SM d321 B	6.5523	0.000424	0.015247	COPD-F vs. COPD-M; COPD-F vs.CON-M; CON-F vs.CON-M
SM d342A	6.5343	0.000434	0.015247	COPD-F vs.CON-M; COPD-F vs.CON-M; CON-F vs.CON-M
SM d423 A	5.9594	0.000869	0.026405	COPD-F vs.CON-M; CON-F vs.CON-M
SM d342B	5.9524	0.000876	0.026405	COPD-F vs.CON-M; CON-F vs.CON-M
SM d371 B	5.7273	0.001152	0.032411	COPD-F vs.CON-M; CON-F vs. COPD-M; CON-F vs.CON-M
PC 343 A	5.6496	0.001267	0.033407	COPD-F vs.CON-M; CON-F vs.CON-M
SM d392 B	5.5296	0.001467	0.033769	COPD-F vs. COPD-M; COPD-F vs.CON-M; CON-F vs. COPD-M; CON-F vs.CON-M
PC 352 A	5.5039	0.001514	0.033769	COPD-F vs.CON-M; CON-F vs.CON-M
SM d363 B	5.4571	0.001603	0.033769	COPD-F vs.CON-M; CON-F vs.CON-M
SM d391	5.4289	0.001659	0.033769	COPD-F vs. COPD-M; COPD-F vs.CON-M; CON-F vs.CON-M
Ceramide d321	5.3837	0.001754	0.033769	COPD-F vs.CON-F; COPD-F vs.CON-M; COPD-M vs.CON-M; CON-F vs.CON-M
FA 141	5.3805	0.001761	0.033769	COPD-F vs. COPD-M; COPD-F vs.CON-F; COPD-F vs.CON-M

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SM d423 B	5.2575	0.002048	0.037568	COPD-F vs.CON-M; CON-F vs. COPD-M; CON-F vs.CON-M
FA 161	5.2165	0.002153	0.037862	COPD-F vs. COPD-M; COPD-F vs.CON-F; COPD-F vs.CON-M

M, male; F, Female; FDR, False discovery rate; COPD, chronic obstructive pulmonary disease; CON, controls.

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Supplementary Table SDC 2. Sphingomyelin (SM) metabolite concentrations that were significantly different between males and females in the smoker control group.

	T value	P value	FDR
SM d322 A	5.0406	4.42E-06	0.000207
SM d322 B	4.9329	6.57E-06	0.000207
SM d403	4.2554	7.30E-05	0.001534
SM d423 A	4.0718	0.000137	0.002151
SM d412 A	3.9271	0.000221	0.00243
SM d363 A	3.9139	0.000231	0.00243
SM d402 A	3.8024	0.000334	0.002496
SM d371 B	3.7873	0.000351	0.002496
SM d412 B	3.782	0.000357	0.002496
SM d363 B	3.7262	0.000427	0.002692
SM d342 A	3.6856	0.000487	0.002788
SM d423 B	3.6023	0.000635	0.003135

SM d301 A	3.5964	0.000647	0.003135
SM d342 B	3.5445	0.000762	0.003428
SM d321 A	3.3925	0.001221	0.005128
SM d362 A	3.3449	0.001412	0.005415
SM d371 A	3.3336	0.001461	0.005415
SM d321 B	3.2448	0.00191	0.006683
SM d412 C	3.1006	0.002922	0.009211
SM d362 B	3.0916	0.003	0.009211
SM d320 A	3.0836	0.00307	0.009211
SM d382 A	2.9913	0.004004	0.011466
SM d392 B	2.971	0.004242	0.01162
SM d301 B	2.9399	0.004633	0.011918
SM d391	2.9325	0.004729	0.011918
SM d331 A	2.8927	0.005288	0.012814
SM d382 B	2.7557	0.007712	0.017995
SM d340 A	2.7387	0.008075	0.018169
SM d331 B	2.6503	0.01023	0.022224
SM d402 B	2.5422	0.013571	0.028499
SM d361 A	2.4758	0.016087	0.032692

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SM d432 A	2.4614	0.016685	0.032729
SM d402 A	2.4506	0.017144	0.032729
SM d422 A	2.4276	0.018164	0.033641
SM d432 B	2.4163	0.018689	0.033641
SM d361 B	2.3764	0.020638	0.036116
SM d340 B	2.3105	0.024257	0.041302
SM d392 A	2.2419	0.028615	0.04744

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Supplementary Table SDC 3. Sphingomyelin (SM) metabolite concentrations that were significantly different between males and females in the COPD group.

	T value	P value	FDR
SM d322 A	4.450	0.00006	0.00391
SM d301 A	3.769	0.00051	0.01593
SM d322 B	3.618	0.00079	0.01663
SM d301 B	3.264	0.00219	0.03445